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Importance of tyrosine phosphorylation in receptor kinase complexes

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Tyrosine phosphorylation is an important post-translational modification that is known to regulate receptor kinase (RK)-mediated signaling in animals. Plant RKs are annotated as serine/threonine kinases, but recent work has revealed that tyrosine phosphorylation is also crucial for the activation of RK-mediated signaling in plants. These initial observations have paved the way for subsequent detailed studies on the mechanism of activation of plant RKs and the biological relevance of tyrosine phosphorylation for plant growth and immunity. In this Opinion article we review recent reports on the contribution of RK tyrosine phosphorylation in plant growth and immunity; we propose that tyrosine phosphorylation plays a major regulatory role in the initiation and transduction of RK-mediated signaling in plants.

Receptor kinases

RKs are plasma membrane-localized proteins that initiate signaling in a multitude of plant processes. RKs are composed of an ectodomain potentially involved in ligand binding, a single-pass transmembrane domain, and an intracellular kinase domain. Well-studied examples of RK-mediated signaling pathways are those triggered by plant perception of the steroid hormones brassinosteroids (BRs), which promote plant growth [1], or of pathogen-associated molecular patterns (PAMPs), leading to immune responses that constitute the first active layer of plant immunity [2].

Plant RKs have traditionally been classified as serine/threonine kinases [3]. This is in contrast with most animal RKs where tyrosine phosphorylation is important for their activation and subsequent signaling, leading to the onset of a multitude of cellular processes [4,5]. Phosphorylation on tyrosine residues was originally observed in plant RKs after autophosphorylation *in vitro* [6–8], although until recently it was not clear whether this also occurred *in vivo*. Reports over the past 5 years have shown that, in addition to serine/threonine phosphorylation, several RKs in *Arabidopsis thaliana* (hereafter referred to as *Arabidopsis*) can undergo tyrosine phosphorylation, and that this modification plays an important role in their activation and in the initiation of subsequent signaling [8–10] (Figure 1, Table 1). In addition, RK-associated proteins are also regulated by tyrosine phosphorylation, uncovering an additional role of this post-translational modification in downstream signal transduction [11–13] (Figure 1; Table 1). In this Opinion article we propose that tyrosine phosphorylation plays a major role in the activation, transduction, and, potentially, specificity of RK-mediated signaling in plants.

Tyrosine phosphorylation in the BR receptor complex

The BR signaling pathway is one of the best studied in plants [1]. In a seminal article published by Oh and coworkers [8] it was observed that the BR receptor, the leucine-rich repeat (LRR)-RK BRASSINOSTEROID-INSENSITIVE 1 (BRI1), is able to autophosphorylate on tyrosine residues, in addition to serine and threonine residues [8]. This was unexpected because BRI1 was classified as a serine/threonine kinase, alongside other plant RKs [14,15]. Strikingly, the authors also reported an unanticipated difficulty in the identification of specific BRI1 phosphorylated tyrosines *in vivo* when using liquid chromatography–tandem mass spectrometry (LC–MS/MS). However, through a thorough analysis using site-directed mutagenesis and modification- and sequence-specific antibodies, the authors identified a single tyrosine residue in the juxtamembrane domain of BRI1 (Y831; Figure 1; Table 1) which plays an important role in BR signaling *in vivo*, without affecting overall BRI1 kinase activity [8]. Additional tyrosine residues, Y956 and Y1072, were found to be phosphorylated *in vivo* and *in vitro*, respectively, although mutations of these residues abolished BRI1 kinase activity [8] (Figure 1; Table 1).

Moreover, it was observed that the LRR-RK BRI1-ASSOCIATED KINASE 1 (BAK1), which acts as a BR coreceptor with BRI1 [16], was able to autophosphorylate on tyrosine residues, and this prompted a follow-up study on the relevance of different tyrosine residues in BAK1 [10]. Using site-directed mutagenesis and modification- and sequence-specific antibodies, a single tyrosine residue in the C-terminal domain of BAK1 (Y610; Figure 1; Table 1) was identified as a major autophosphorylation site upon

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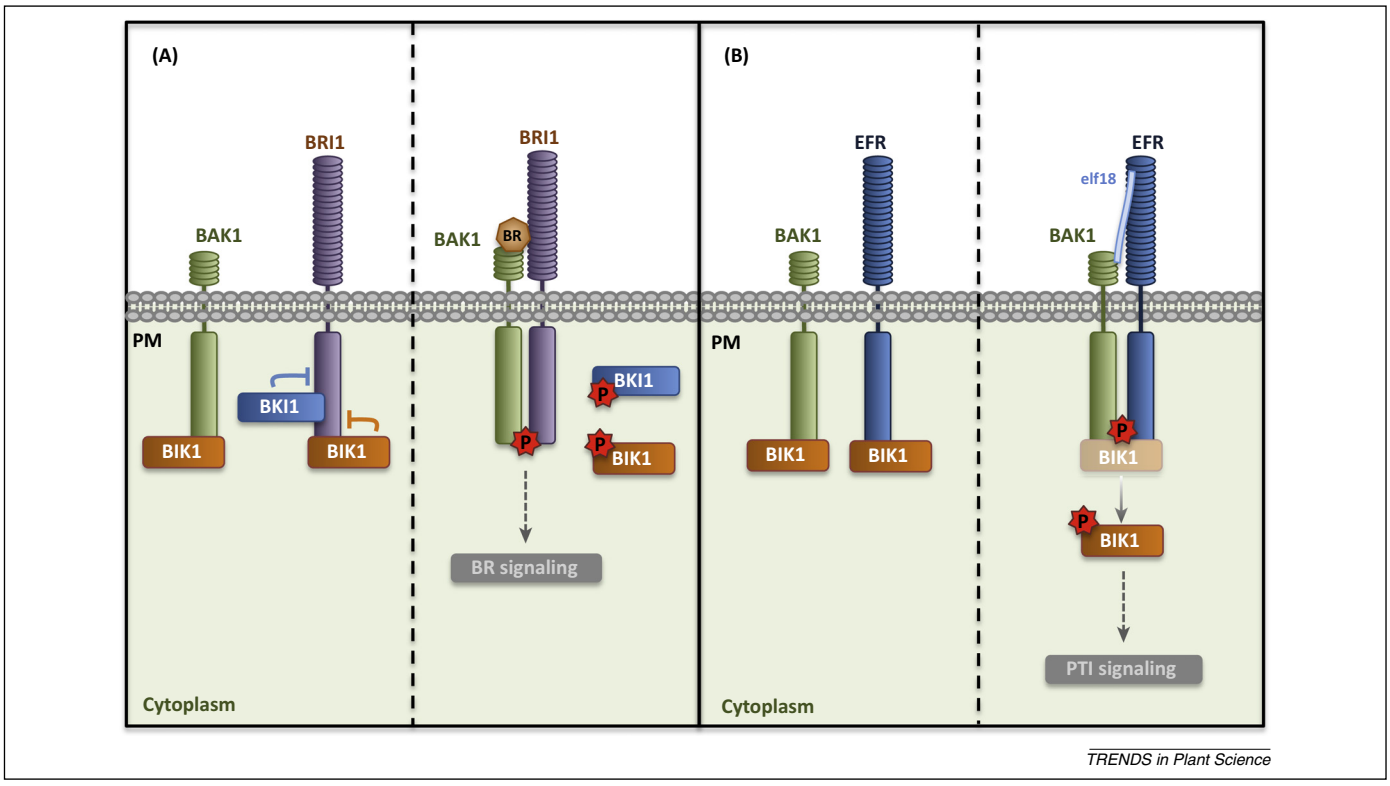

Figure 1. Simplified diagram of tyrosine-phosphorylated proteins involved in early brassinosteroid (BR)- and PAMP (here, elf18)-triggered signaling. **(A)** In the absence of BR, BKI1 and BIK1 negatively regulate the activation of BRI1. BR perception leads to phosphorylation (P) of BKI1 Y211, followed by the release of the protein into the cytosol, which relieves the inhibition of BRI1 kinase activity and increases the affinity between BRI1 and BAK1 kinase domains. BR perception also leads to the release of BIK1. The dissociation of both negative regulators allows the formation of a fully-active BR receptor complex. **(B)** BIK1 associates with EFR and BAK1 in unelicited conditions. Upon elf18 perception, EFR Y836 is phosphorylated and contributes to the ligand-induced activation of the EFR–BAK1 complex. PAMP perception also leads to the phosphorylation of BIK1, which is released from the receptor complex and activates downstream immune responses. Abbreviations: BAK1, BRI1-ASSOCIATED KINASE 1; BIK1, BOTRYTIS-INDUCED KINASE 1; BKI1, BRI1-KINASE INHIBITOR 1; BRI1, BRASSINOSTEROID-INSENSITIVE 1; EFR, EF-TU RECEPTOR; PAMP, pathogen-associated molecular pattern; PM, plasma membrane.

BR perception that is required for BR-triggered signaling [10].

Together, these two reports unequivocally demonstrated that both BRI1 and BAK1 are dual-specificity kinases, and that tyrosine phosphorylation plays a prominent role in the activation of the BR receptor complex and subsequent signal transduction.

Later work added more complexity to the role of tyrosine phosphorylation in the regulation of BR receptor activation. One of the mechanisms that keeps BRI1 in an inactive state

Table 1. Tyrosine-phosphorylated residues in components of RK complexes[a]

Protein	Residue	Identification	Ligand-dependency	Auto-/trans-phosphorylation	Evidence of phosphorylation	Mutation to Phe abolishes kinase activity			Refs
						Detection by LC/MS-MS	Sequence- and phosphorylation-specific antibodies	Site-directed mutagenesis	
BRI1	Y831	*In vivo* *In vitro*	Nd	Auto	Yes	Yes	Yes	No	[7]
	Y956	*In vivo* *In vitro*	Nd	Auto	No	Yes	Yes	Yes	[7]
	Y1072	*In vitro*	Nd	Auto	No	Yes	Yes	Yes	[7]
BAK1	Y610	*In vivo* *In vitro*	BL-induced	Auto	No	Yes	Yes	No	[9]
BKI1	Y211	*In vivo*	BL-induced	Trans	No	No	Yes	N/A	[10]
EFR	Y836	*In vivo*	elf18-induced	Nd	Yes	No	Yes	No	[8]
BIK1	Y23	*In vitro*	Nd	Auto	Yes	No	No	No	[11]
	Y150	*In vitro*	Nd	Nd	No	No	Yes	Yes	[11]
	Y168	*In vitro*	Nd	Auto/trans	Yes	No	No	No	[12,33]
	Y214	*In vitro*	Nd	Auto/trans	Yes	No	Yes	No	[12,33]
	Y234	*In vitro*	Nd	Auto	Yes	No	Yes	No	[11]
	Y243	*In vitro*	Nd	Auto/trans	No	No	Yes	No	[11]
	Y250	*In vitro*	Nd	Auto/trans	Yes	No	Yes	No	[11,12,33]

[a]Abbreviations: BAK1, BRI1-ASSOCIATED KINASE 1; BIK1, BOTRYTIS-INDUCED KINASE 1; BKI1, BRI1-KINASE INHIBITOR 1; BRI1, BRASSINOSTEROID-INSENSITIVE 1; EFR, EF-TU RECEPTOR. Nd, not determined.